Supporting Information for

Side-Chain Proline-Based Polymers as Effective Inhibitors for *in vitro* Aggregation of Insulin

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Synthesis of Boc-Pro-HEMA monomer. *Tert*-butoxycarbonyl (Boc)-proline methacryloyloxyethyl ester (Boc-Pro-HEMA) monomer was synthesized by condensation reaction of Boc protected proline (Boc-L-Pro-OH) with 2-hydroxyethyl methacrylate (HEMA) in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), following a previous literature report.[1] Typically, Boc-L-Pro-OH (5.0 g, 23.23 mmol) dissolved in dry dichloromethane (DCM) (100 mL) was taken in a 250 mL round bottom flask equipped with a magnetic stir bar and kept on ice-water bath. Then a solution of DCC (5.75 g, 27.88

mmol) and DMAP (0.58 g, 4.65 mmol) in DCM (50 mL) was added dropwise to the reaction mixture under continuous stirring. Next, HEMA (3.0 g, 23.23 mmol) was added dropwise and ice-water bath was removed after 30 min. The reaction mixture was then allowed to stir for 24 h at room temperature. Next, the reaction mixture was filtered to remove insoluble *N*,*N*'-dicyclohexylurea (DCU) and the organic layer was washed with 1.0 N HCl solution, saturated $NaHCO_3$ solution followed by brine solution and dried over anhydrous Na_2SO_4. Finally the resulting crude product was purified by silica gel column chromatography to obtain pure Boc-Pro-HEMA monomer. The monomer was characterized by ^{1}H NMR and ESI-MS spectroscopy. The ^{1}H NMR spectrum of Boc-Pro-HEMA monomer exhibited typical vinyl proton signals at 6.1 and 5.6 ppm (Figure S1). From ESI-MS analysis, the experimental molecular mass (*m*/*z* for [Boc-Pro-HEMA + Na^+]) was found to be 349.82 which matches nicely with the theoretical value 350.16.

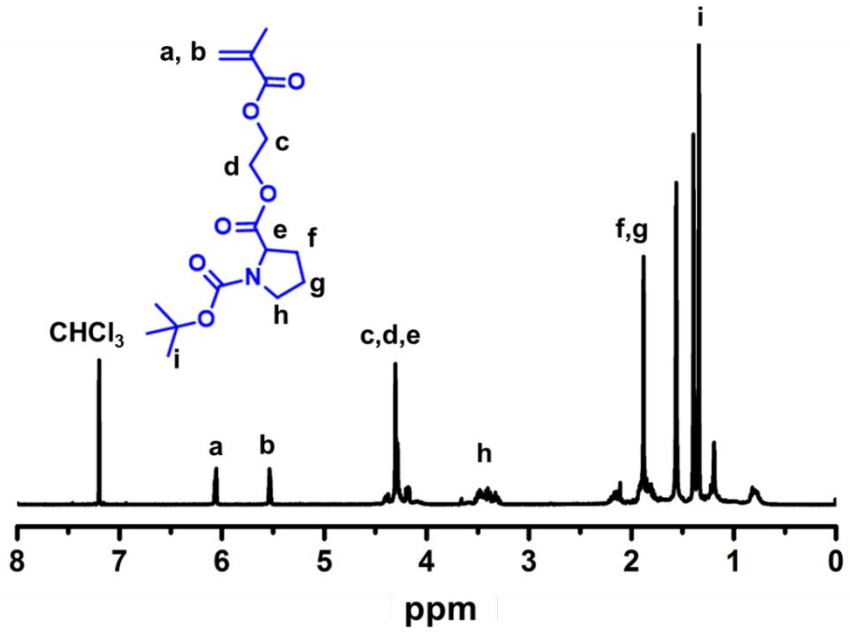

Figure S1. ^{1}H NMR spectrum of Boc-Pro-HEMA monomer in $CDCl_3$.

Dynamic light scattering (DLS) measurements. The particle size distribution of insulin aggregates in absence and presence of different polymers was determined by DLS study. Samples were diluted in Milli-Q water and size distributions were measured using a Malvern Nano ZS instrument.

Transmission electron microscopy (TEM). The morphology and growth of insulin fibrils in absence and presence of different polymers were analyzed by TEM technique. The stock solution of insulin fibrils and polymer treated insulin fibrils were diluted 100 fold. A drop of sample was then placed on a carbon coated TEM grid, air dried and the images were acquired.

Fluorescence microscopy. Fluorescence microscopy was used to understand the morphology of insulin fibrils in absence and presence of polymers. Briefly, 5 μL of samples were mixed with 10 μL of ThT (1 mM) and incubated for 15 min. One drop of sample was then placed on a glass slide, covered with a cover slip and images were captured under FITC filter in optical microscope (BX53, Olympus) connected with a CCD camera.

Circular dichroism (CD) study. The secondary structural alterations of insulin fibrils in absence and presence of polymers were analyzed by Far-UV CD spectroscopy using a 0.1 cm path length cell at 25 °C. Spectra were recorded in the region 190-240 nm with a response time of 4 s and scan speed of 50 nm/min. Three scans were accumulated for each spectrum. The secondary structure was analyzed using DICHROWEB, an online server for protein secondary structure analysis from CD spectroscopic data.

Isothermal titration calorimetry (ITC). The interaction behavior of insulin fibrils with polymers was studied by using isothermal titration calorimeter (ITC (MICROCAL PEAQ-ITC, Malvern Instruments)). Polymers were taken into Hamilton syringe and titrated into the sample cell containing native insulin and insulin fibrils. The reference cell was filled with the same

buffer. The experiments were designed for a total of 19 consecutive injections. During titration 2 µL of 1 mM concentration of polymer was injected to native insulin or insulin fibrillar solution containing sample cell. The duration between consecutive injections was 10 s with an interval of 4 min between each injection. The ITC profiles were analyzed using Microcal PEAQ-ITC software to determine the heat of interaction as well as thermodynamic parameters.

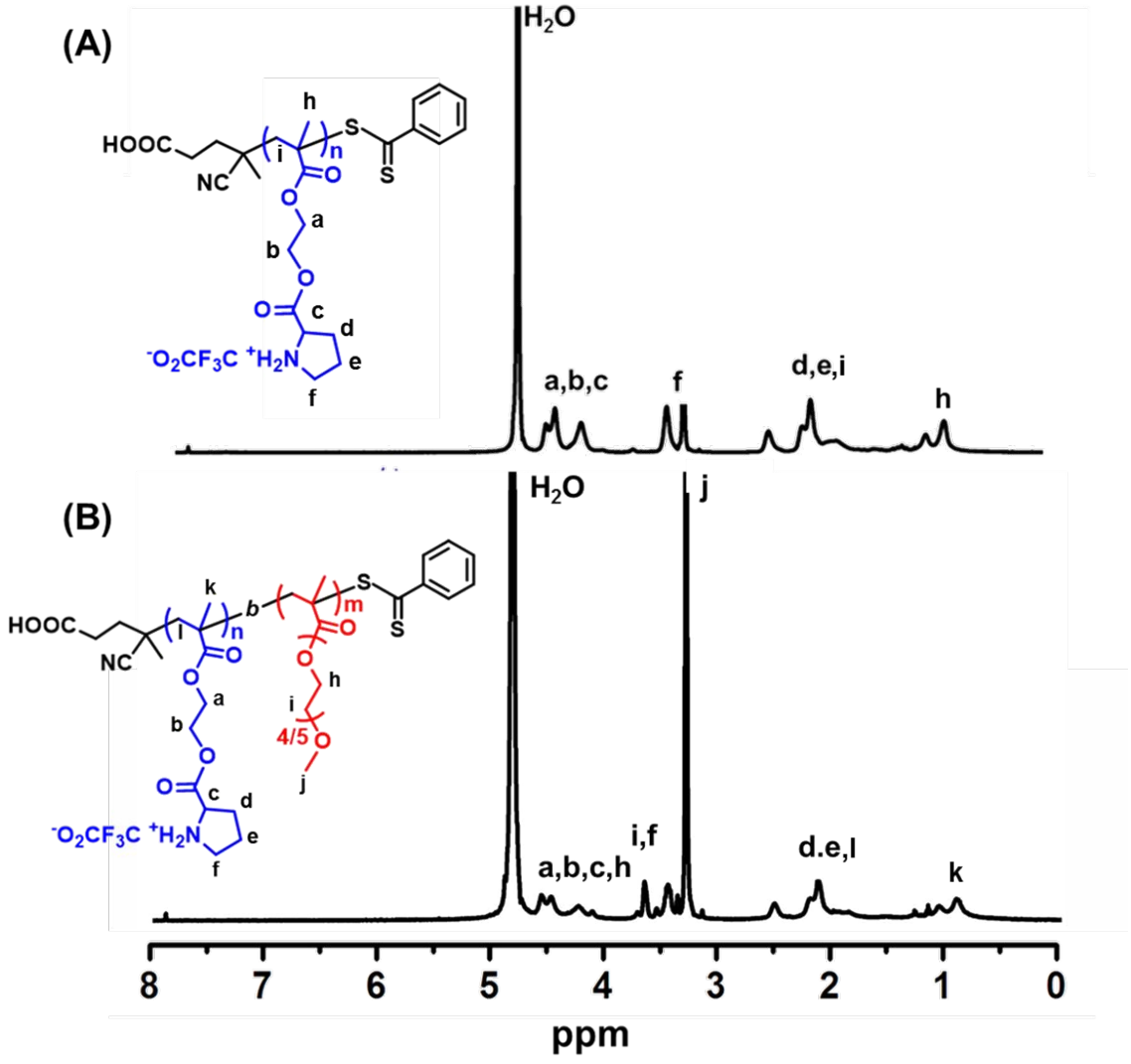

Figure S2. ^{1}H NMR spectra of (A) P(Pro-HEMA) and (B) P(Pro-HEMA)-*b*-PPEGMA-1 in D_2O.

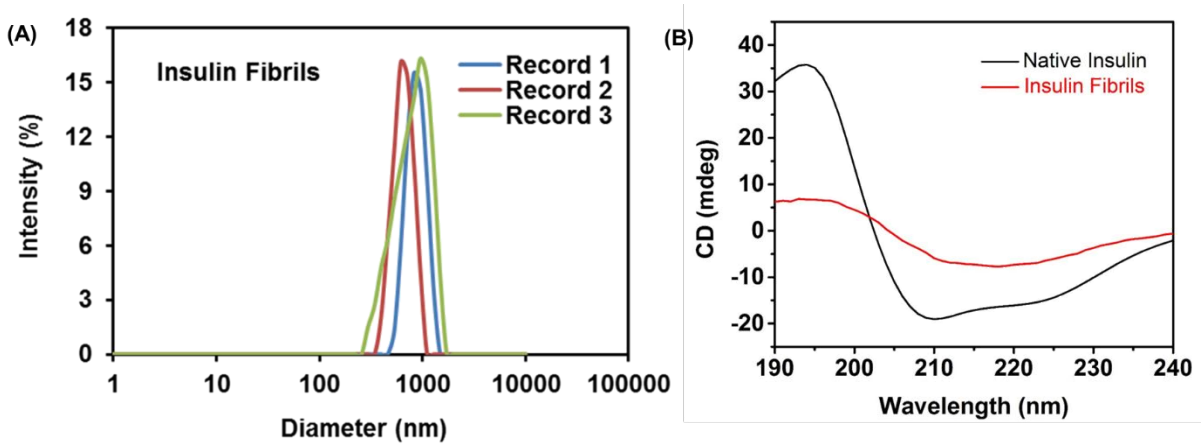

Figure S3. (A) Size distribution of insulin fibrils and (B) Far-UV CD spectra of native insulin and insulin fibrils.

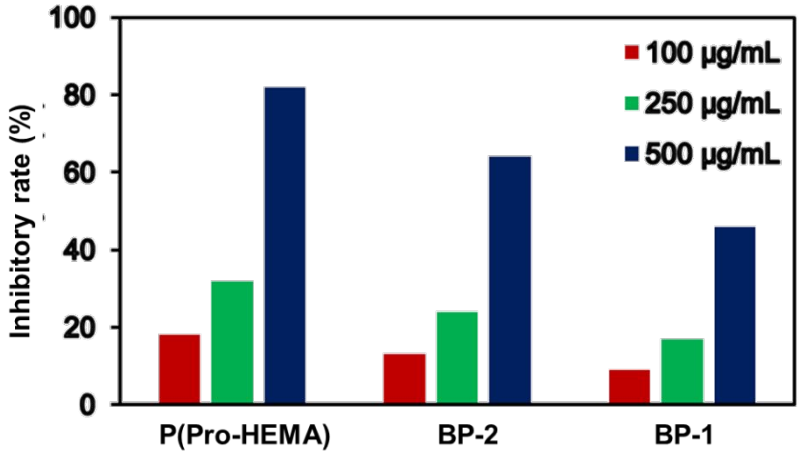

Figure S4. Histogram of inhibitory rate at different concentrations of polymers on insulin fibrillation pathway.

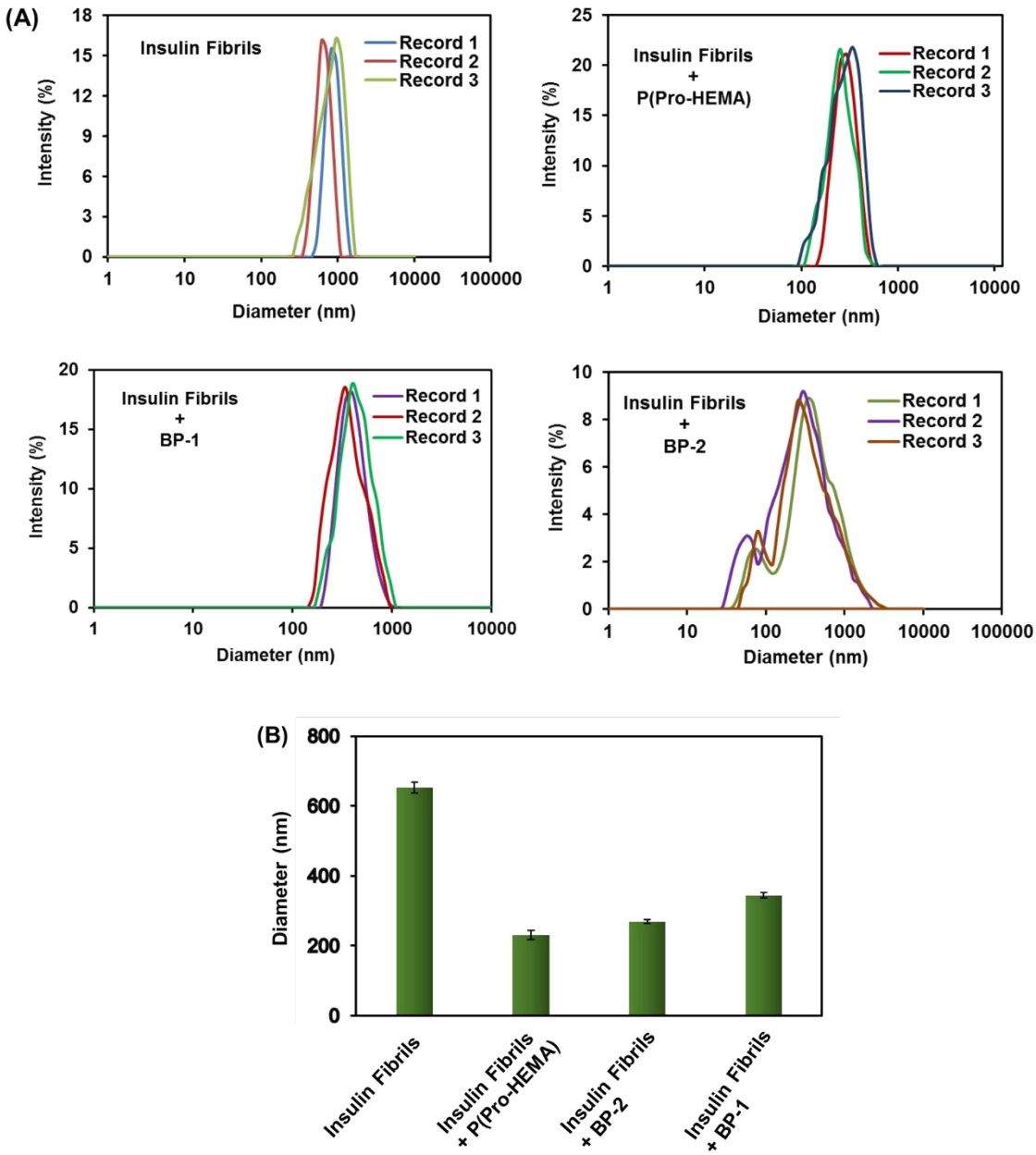

Figure S5. (A) Size distribution profiles of insulin fibrils in absence and presence of polymers and (B) histogram of average sizes of insulin fibrils in absence and presence of polymers.

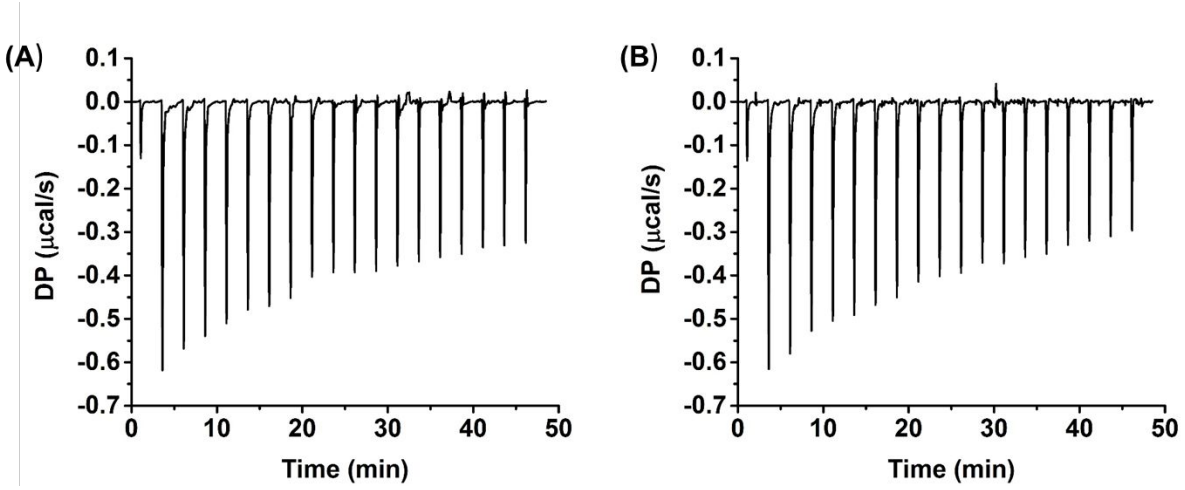

Figure S6. ITC profile of (A) native insulin with BP-1 and (B) insulin fibrils with BP-1.

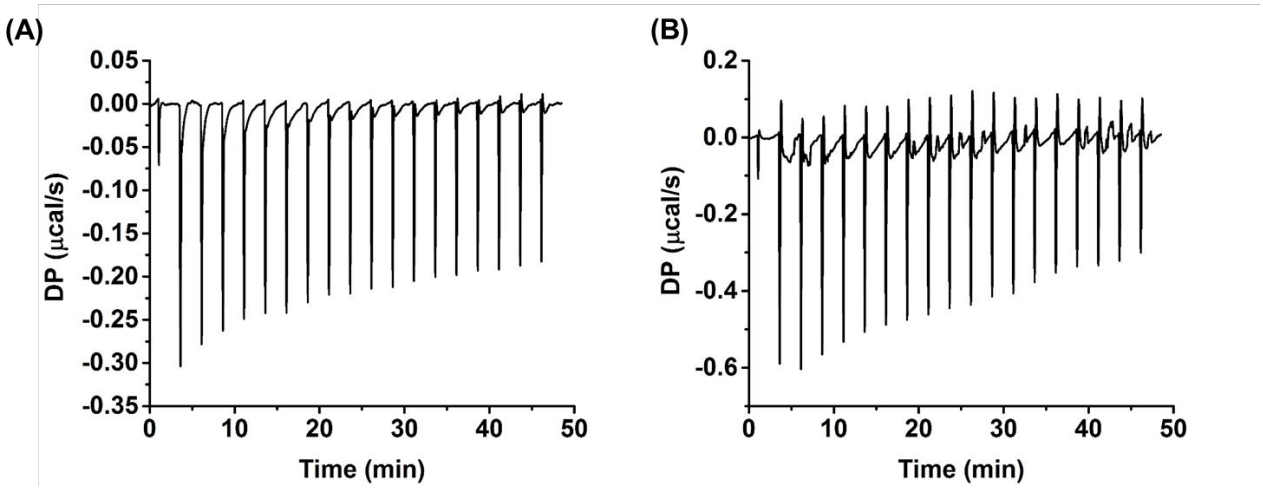

Figure S7. ITC profile of (A) native insulin with BP-2 and (B) insulin fibrils with BP-2.

References

(1) Maiti, B.; Ruidas, B.; Roy, S. G.; De, P. RAFT Polymerization of Side-Chain L-Proline Containing Methacrylate Monomer: Controlled Synthesis, Thermoresponsiveness and Self-Assembly. *In Nanospectrum: A Current Scenario*; Chakrabarti, S., Mukherjee, P., Khan, G. G., Adhikary, A., Patra, P., Bal, J. K., Eds.; Allied Publishers Pvt. Ltd., 2015; pp. 41-48.